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Unraveling the Warp and Weft of B Cell Fate

Two recent *Immunity* articles (Enzler et al., 2006; Sasaki et al., 2006) probe the roles of Nuclear Factor κ-B (NF-κB) pathways in survival and differentiation mediated by B cell activation factor of the TNF family (BAFF).

The characterization of B cell activation factor of the TNF family (BAFF, also termed BLyS) and its principal receptor, BAFF-R, has provoked reassessment of the notion that B cell receptor (BCR) signaling alone governs B cell survival and selection. Indeed, BAFF-R mutations yield inordinate death among primary B cells despite functional BCRs (Harless et al., 2001); and excess BAFF fosters humoral autoimmunity in spite of normal BCR diversity (Mackay et al., 1999). Nonetheless, the BCR is equally critical to B cell survival (Lam et al., 1997), and the outcome of BAFF signaling varies in the context of both BAFF availability and BCR specificity (Lesley et al., 2004; Thien et al., 2004). Thus, probing BAFF signal transduction is a key first step towards revealing why these two systems, while both necessary, operate properly only in concert.

The nuclear factor-κB (NF-κB) family is an attractive starting point for interrogation because its members act downstream of several B cell surface molecules, including CD40, Toll-like receptors (TLRs), and the BCR (Figure 1). At least two pathways can initiate formation of active NF-κB transcriptional regulators. The classical (NF-κB1) pathway involves kinase cascades that degrade inhibitory complexes, affording nuclear localization of active dimers; whereas the nonclassical (NF-κB2) pathway regulates the formation of p52 via p100 degradation. Prior studies suggest that both pathways are activated by BAFF (Hatada et al., 2003), and knockouts for either NF-κB essential modulator (NEMO) or for NF-κB-inducing kinase (NIK)—upstream components of NF-κB1 and NF-κB2 pathways, respectively—show reduced B cell numbers reminiscent of BAFF-R defects. Accordingly, two recent Immunity articles (Enzler et al., 2006; Sasaki et al., 2006) have employed powerful gene-knockout and transgenic strategies to probe the role of each NF-κB pathway in BAFF-mediated survival and selection.

Citing the similarities between NEMO- and BAFF-R-deficient phenotypes, Sasaki et al. reason that enforced NF-κB1 activity should circumvent BAFF-R defects and restore B cell survival. They test this idea by characterizing mice in which a constitutively active IκB Kinase 2 (IKK2ca) is expressed in mice lacking BAFF-R. These mice exhibit B cell subset distributions similar to those of BAFF-R-sufficient controls, and their B cells display characteristics associated with BAFF-R function, such as nuclear exclusion of protein kinase-Cδ (PKC-δ) and upregulation of Bcl-2 family members including Bcl-xl and A1. Nonetheless, several observations hint at additional requirements for optimal BAFF signaling. First, as the authors note, NEMO deficiency yields a milder B cell phenotype than does BAFF-R deficiency. Second, IKK2ca expression in BAFF-R-sufficient mice yields about twice as many B cells as it does in the BAFF-R knockouts, suggesting BAFF-R per se provides additional, non-redundant signals. Finally, BCR signaling remains necessary for fully appropriate responses to this arm of the BAFF-R cascade; IKK2ca fails to rescue marginal-zone development in mice lacking the BCR coreceptor component CD19.

Enzler et al. not only examine survival but also exploit the emergence of autoreactivity as a measure of BAFF-R activity in vivo. Consistent with Sasaki et al., they find that BAFF-R activates the NF-κB1 pathway and influences BAFF-mediated survival in vitro. However, they find that NF-κB2 is necessary for optimal survival, as well as for maximal upregulation of *Bcl-2* gene family members. Moreover, they observe a strict NF-κB2 requirement for induction of the pro-survival kinase Pim-2 and resultant Bad phosphorylation. Thus, although NF-κB1 is clearly important for BAFF-R signaling, NF-κB2 seems necessary for full function. Extending the suggestion of such complementary roles to an in vivo system, they assess how blocking each pathway impacts marginal-zone differentiation and autoantibody formation in BAFF transgenics. They find that BAFF signaling through NF-κB2 upregulates integrins and leads to marginal-zone retention and survival of autoreactive B cells; their findings extend earlier reports that showed

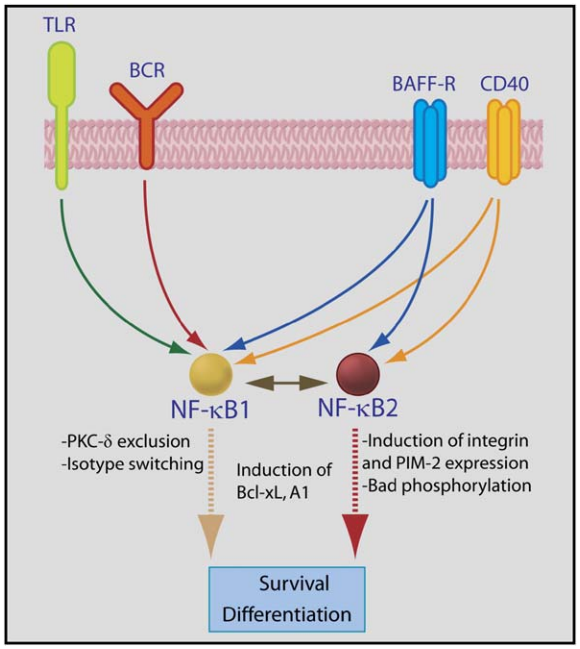

Figure 1. BAFF Acts through Both NF-κB1 and NF-κB2 Pathways to Foster Survival and Differentiation

Both classical and nonclassical NF-κB pathways are activated by BAFF/BAFF-R interactions, leading to the upregulation of gene products key to survival, differentiation, and localization. Although some of these actions seem to be limited to either NF-κB1 or NF-κB2, others appear to be under synergistic control. These pathways also lie downstream from BCR signaling, which along with BAFF is essential for B cell survival, as well as downstream from several other B cell ligand-receptor systems. Together, this growing list suggests a general model whereby exogenous cues are integrated to yield apposite survival and differentiation responses.

that autoreactive clonotypes in BCR transgenics survive in excess BAFF and that their admission to the marginal zone is critically dependent on BAFF availability (Lesley et al., 2004; Thien et al., 2004). Although not required for these effects, NF-κB1 seems necessary for the appearance of isotype-switched serum autoantibodies.

This emerging picture suggests that a model of symbiotic crosstalk between the two NF-κB pathways and their upstream activators may reconcile otherwise perplexing disparities. For example, Sasaki et al. show that constitutive NF-κB1 activation yields increased p100 and thus provides a key substrate for the NF-κB2 pathway. However, in the absence of BAFF-R, p52 generation is curtailed despite these elevated p100 levels. Thus, the synergistic effects reported by Enzler et al., as well as the additional effectiveness of BAFF-R when it is present with IKK2ca, might reflect a role for NF-κB1-dependent p100 generation in sustaining BAFF-R-driven NF-κB2 activation. Extending this notion of crosstalk to other B cell surface molecules that stimulate NF-κB pathways has potentially exciting ramifications. It may disclose how BCR signaling through NF-κB1 can govern NF-κB2-dependent aspects of BAFF-R function; for example, such aspects might include positive selection and marginal-zone differentiation. Indeed, BCR signaling strength governs both of these events, but the thresholds vary with BAFF availability. Similarly, such crosstalk may begin to explain how CD40 costimulation, which strongly activates both NF-κB pathways, can alone mediate survival and differentiation, whereas innate activation receptors that sidestep costimulation yield a different and more limited subset of these outcomes. Probing the exact nature of this crosstalk will require interrogating the upstream molecular connections between these pathways and key B cell activation and regulatory receptors, as well as determining how the balance of these signals impacts the mix of downstream transcriptional regulators.

Thus, these papers together set the stage for unraveling the molecular weaving of B cell fate by suggesting mutually supporting roles for NF-κB1 and NF-κB2 pathways in BAFF signaling (Figure 1). Understanding this relationship should prove key to revealing how BAFF regulates B cell survival and differentiation in a context-dependent manner. It likely includes semiautonomous roles for each pathway, such that signals diverge downstream of BAFF-R to regulate survival and differentiation. In addition, it provides a fabric within which signals from BAFF-R, the BCR, and other receptors upstream of NF-κB pathways can interweave and thus allow survival and differentiation to be guided by the aggregate of these inputs.

Jason E. Stadanlick[1] and Michael P. Cancro[1]
[1] 284 John Morgan
Department of Pathology and Laboratory Medicine
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104

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